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Paris, 06 AUGUST 1999

For the Director General of the National
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The Head of the Patents Department

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REQUEST FOR GRANT

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2 APPLICATION Nature of intellectual property right
Patent of Invention

Search Report Immediate

Title of invention (200 characters maximum)

Use of new agents which induce cell death in synergy with interferons

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PATENT OF INVENTION, REGISTRATION CERTIFICATE

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NATIONAL REGISTRATION No.

98 09886

TITLE OF THE INVENTION:
Use of new agents which induce cell death in synergy with interferons

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DOCUMENT COMPRISING MODIFICATIONS

PAGES OF THE DESCRIPTION OR CLAIMS OR DRAWING SHEET(S)			M. C.	DATE OF CORRESPONDENCE	DATE STAMP OF CORRECTING OFFICIAL
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A change made to the wording of the original claims, unless it follows from the provisions of Article R.612-36 of the Intellectual Property Code, is indicated by "M.C." (modified claims)

INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE
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The use of new agents which induce cell death
in synergy with interferons

ABSTRACT OF THE TECHNICAL CONTENT OF THE INVENTION

The present invention relates to the use of new agents which induce cell death, particularly an agent which enables superexpression of the PML protein on nuclear bodies, in association with interferons, to induce the death of undesirable cells.

No Figure

The present invention relates to the use of new agents which induce cell death, particularly an agent which enables superexpression of the PML protein on nuclear bodies, in association with interferons, to induce the death of undesirable cells.

Nuclear bodies are structures of unknown function which are associated with the nuclear matrix, and contain a certain number of proteins, including PMLI Spl00, ISG20, PIC-1/SUMO 1, lyspl00, PLZF, Int-6, CBP, Rb, RFP and ribosomal protein P (Lamond et al., 1998). The gene which codes for the PML protein (for "promyelocytic leukaemia") has been identified from its fusion with the *RARα* gene (nuclear receptor of retinoic acid) in the t(15;17) translocation found in patients affected by acute promyelocytic leukaemia (APL). This PML gene is a target gene of interferons, and its superexpression causes a stoppage of growth of some cell lines (Koken et al., 1995). In malignant APL cells, the PML protein is not located on nuclear bodies but is relocated due to the expression of PML-RARα. Arsenic oxide induces the return of PML to its normal location, as well as the death of the cell. In normal non-APL cells, where the PML location is normal, arsenic induces aggregation of PML to form large, modified bodies, but this phenomenon is not accompanied by cell death (Zhu et al., 1997).

The authors of the present invention have now discovered that superexpression of the PML protein located on nuclear bodies causes cell death by an original mechanism which is different from that of caspase-induced apoptosis.

The main consequence of this discovery is that a substance which promotes PML protein addressing to cell bodies, and/or its stabilisation, is particularly useful for inducing the death of undesirable cells.

Said substances which induce PML protein addressing to nuclear bodies, and/or its stabilisation, can be identified by standard tests known to one skilled in the art. In particular, measurement of the intracellular transit between cytoplasmic and nucleoplasmic fractions and the fraction associated with nuclear bodies and the stabilisation of the PML protein can be made by Western blotting.

In particular, said undesirable cells can be tumour cells, cells infected by a virus, a parasite or a bacterium, immune cells participating in an inappropriate immune reaction, genetically modified cells, or senescent or hyperplasic cells.

A "tumour" should be understood to include any undesirable benign or malignant proliferation of cells, including solid cancers and leukaemias and lymphomas in particular. Malignant tumours include chronic myeloid leukaemias and melanomas in particular.

The present invention therefore relates to the use of at least one substance which promotes PML protein addressing to nuclear bodies and/or its stabilisation for the production of a drug intended to induce the death of undesirable cells, with the exception of leukaemia cells of acute promyelocytic leukaemia when said substance is a compound of arsenic.

Since expression of the PML protein is induced by interferons, the presence of interferons, whether of endogenous origin or administered to the patient simultaneously or sequentially, is necessary for the efficacy of the envisaged treatment.

Surprisingly, the authors of the present invention have discovered in particular that zVAD (benzyloxycarbonyl-Val-Ala-Asp(O-methyl)-fluoromethylketone) firstly stabilises the PML protein and secondly accelerates cell death induced by interferons.

However, zVAD was initially known as an inhibitor of caspases, which are proteases involved in the apoptosis process (Salvesen et al., 1997). Studies (McCarthy and al., 1997) have also shown that zVAD strongly inhibits or delays cell death. The discovery made by the authors of the present invention, according to which zVAD does not block cell death induced by interferons but on the contrary accelerates it, is therefore contrary to the result which one skilled in the art would expect.

The present invention relates in particular to the use of an inhibitor and/or caspase substrate such as zVAD, for the production of a drug intended to induce the death of undesirable cells.

The term "caspase substrate" should be understood to include all compounds capable of binding to caspases.

The authors of the present invention have also discovered that arsenic, and more particularly arsenic trioxide, firstly promotes the addressing of the PML protein to cellular bodies and secondly accelerates cell death induced by interferons.

The present invention more particularly relates to the use of a compound of arsenic or a compound having the same biological properties as arsenic for the production of a drug intended for inducing the death of undesirable cells, with the exception of the leukaemia cells of acute promyelocytic leukaemia.

Amongst compounds of arsenic, arsenic trioxide should be mentioned in particular.

The term "compound having the same biological properties as arsenic" should be understood to mean any compound which, like arsenic, is a phosphatase inhibitor and/or is capable of creating covalent adducts by bonding with dithiol groupings.

Inhibitors and/or caspase substrates or compounds of arsenic or compounds having the same biological properties as arsenic are preferably used for inducing the death of undesirable cells in association with the PML protein and/or with an agent which induces superexpression of the protein. Amongst agents which induce superexpression of the PML protein, an interferon is preferably used, such as interferon α or β.

In fact, the authors of the present invention have discovered in particular that caspase substrates, particularly zVAD, as well as compounds of arsenic, particularly arsenic trioxide, act synergistically with interferons to induce and to accelerate cell death.

The simultaneous or sequential administration of PML or of an agent which induces superexpression of the PML protein, such as interferons, can be useless if the amount of PML or of the agent which induces superexpression of the PML protein, such as interferons, of endogenous origin, is sufficient. Nevertheless, according to one preferred embodiment of the invention, the administration of a substance selected from compounds of arsenic, compounds having the same biological properties as arsenic and caspase inhibitors and/or

substrates, is associated with the simultaneous or sequential administration of PML protein and/or of an agent which induces superexpression of the PML protein, such as interferons.

The use of a substance selected from compounds of arsenic, compounds having the same biological properties as arsenic and caspase inhibitors and/or substrates, in association with an interferon, for inducing the death of undesirable cells forms part of the invention whether this is due to the PML protein induced by said interferon or by another mechanism which is also induced by said interferon.

The present invention also relates to a therapeutic treatment method, in which a therapeutically effective amount of at least one substance selected from compounds of arsenic, compounds having the same biological properties as arsenic and caspase inhibitors and/or substrates, in association with a pharmaceutically acceptable vehicle, is administered to a patient who requires treatment such as this.

A therapeutically effective amount of PML protein, and/or of an agent which induces superexpression of the PML protein, such as an interferon, is preferably also administered, simultaneously or sequentially, to said patient.

The present invention also relates to a pharmaceutical composition containing

1) either at least one inhibitor and/or a caspase substrate, associated with:
   - at least one compound of arsenic or a compound having the same biological properties as arsenic,
   - and/or the PML protein
   - and/or at least one agent which induces superexpression of the PML protein, such as an interferon,

in the presence of a pharmaceutically acceptable vehicle;

2) or at least one compound of arsenic or a compound having the same biological properties as arsenic associated with the PML protein and/or with at least one agent which induces superexpression of the PML protein, such as an interferon, in the presence of a pharmaceutically acceptable vehicle.

The present invention also relates to a kit comprising

a) - a pharmaceutical composition (1) containing at least one inhibitor and/or caspase substrate, in association with a pharmaceutically acceptable vehicle;

- and/or a pharmaceutical composition (2) containing at least one compound of arsenic or a compound having the same properties as arsenic, in association with a pharmaceutically acceptable vehicle; and

b) - a pharmaceutical composition (3) containing the PML protein in association with a pharmaceutically acceptable vehicle;

- and/or a pharmaceutical composition (4) containing at least one agent which induces superexpression of the PML protein, such as an interferon, in association with a pharmaceutically acceptable vehicle;

said pharmaceutical compositions being intended for simultaneous or sequential administration.

The mode of administration and the dosage depend on the disease treated and its state of advance, as well as on the weight, age and sex of the patient.

According to the invention, the formulation of the drugs of the invention enables administration to be effected in particular by an oral, anal, nasal, intramuscular, intradermal, subcutaneous or intravenous route.

The administration dose envisaged can be 1 to 50 mg per day, for example, preferably intravenously, for compounds of arsenic, 1 to 260 mg per kg of body weight of caspase substrates such as zVAD, and 1 to 20 million international units (MIU), preferably 3 to 5 MIU, preferably by an intramuscular or subcutaneous route, per day or every two days, for interferon.

The inventors have also discovered that cell death induced by superexpression of PML protein has features which are different from those of caspase-induced apoptosis. In the case of cell death induced by PML, the characteristic nuclear morphologies typical of apoptosis, such as chromatin condensation and nuclear fragmentation, are not observed.

Moreover, whereas cell death induced solely by interferons exhibits the characteristics of apoptosis, the authors of the present invention have observed that the synergistic association

of zVAD with interferons cause this apoptotic phenotype to disappear, cell death then having characteristics which are different from those of apoptosis.

One of the major consequences of this discovery is the capacity of the undesirable cells killed by the mechanism induced by PML to cause an immune reaction against similar undesirable cells which would have escaped the death caused by the PML protein (Melcher et al., 1998, Nature Medicine, Vol. 4, no. 5, pp. 581 -587).

This property makes the use of a substance selected from compounds of arsenic, compounds having the same biological properties as arsenic, and caspase inhibitors and/or substrates, preferably in association with an interferon, for inducing death of undesirable cells, of particular interest insofar as said substance also induces a subsequent immune reaction against undesirable cells.

It can also be advantageously used for treating *ex vivo* a system of cells capable of containing undesirable cells before administration to a patient, for example a bone marrow preparation intended for a graft for a patient with leukaemia, since a preparation such as this generally contains residual malignant cells. A treatment such as this not only enables thee death of undesirable cells contained in the preparation to be induced, but also enables an immune reaction to be induced which is directed against the undesirable cells present in the patient's body to which the treated cell preparation is administered.

Therefore, the present invention also relates to an *in vitro* method of inducing the death of undesirable cell, comprising the placement of undesirable cells in contact with a substance selected from compounds of arsenic, compounds having the same biological properties as arsenic, and caspase inhibitors and/or substrates, said substance preferably being associated with the PML protein and/or with an agent which induces superexpression of the PML protein, preferably an interferon.

The following examples and Figures illustrate the invention without limiting the scope thereof.

KEY TO THE FIGURES

- Figure 1A illustrates the induction of a 90 kD PML protein in a REF(T)PML clone four hours after exposure to variable concentrations of $ZnCl_2$.

- Figure 1B illustrates a FACS analysis of REF(T)PML cells or control cells, four hours 30 minutes after exposure to 150 µM of $ZnCl_2$. The plots on the left illustrate the DNA content in relation to the size of the cells. The plots on the right illustrate the DNA content as a function of the fluorescence (TUNEL).

- Figure 1C illustrates the cytometric analysis of REF(T)PML cells which were treated or not treated with $ZnCl_2$, with etoposide, or with zVAD caspase inhibitor. Labelling was effected with Annexine V-FITC (plots on the left) or rhodamine 123 (plots on the right). The percentage of apoptotic cells is indicated.

- Figure 2A illustrates the absence of cleavage of PARP at the time of the cell death triggered by PML. The cells were treated with 150 µM of $ZnCl_2$, etoposide or zVAD.

- Figure 2E illustrates the activity of caspase CPP32 determined by cutting DEVD-pNA in REF(T) control cells or in REF(T)PML cells The relative absorbances of three independent determinations are illustrated.

- Figure 3A shows the survival of monocytes treated with 1000 U/ml of IFNα. A representative experiment of five experiments is illustrated. The TUNEL tests demonstrate that the reduction in cell numbers is due to apoptosis.

- Figure 3B illustrates histograms indicating the effect of zVAD (100 µM) 24 hours after it was added. The mean values ± the standard deviations from 11 experiments are illustrated.

- Figure 4A shows that zVAD stabilises PML protein in REF(T) cells.

- Figure 4B shows that interferon α (1000 U/ml) induces rat PML in REF(T)PML cells. The arrows indicate different isoforms of the PML.

EXAMPLES

MATERIALS AND METHODS

Plasmid construction

A SacI-BglII fragment (- 69, +55 base pairs) of the promoter of mouse metallothioneine was inserted in a pKS plasmid and was fused with a BglII-BamHI fragment of a PML cdNA, resulting in the plasmid pKSmMt-PML. For the GFP-PML fusion, the same PML fragment was inserted in the BglII site of the vector pEGFP-1 (Clontech). A retroviral vector expressing PML was also constructed by inserting a full-length cDNA from PML (de Thé et al., 1991) in the EcoRI site of SRαtkneo (Muller et al., 1991).

Cell culture

REFC and MEF(T) cells are embryonic fibroblasts of rats and mice immortalised by an SV40T expression vector. REF(K1) cells are immortalised by a SV40T mutant which does not bind Rb, and F111 cells are spontaneously immortalised 3T3 rat fibroblasts. For clone genesis tests, the cells were transfected with 10 μg of SRαtkneo-PML or SRαtkneo in culture trays of 10 cm diameter and were selected by neomycin (500 μg/ml). To obtain inducible clones, a pool of REF(T) cells was co-transfected with the plasmid pKSmMt-PML and a hygromycin resistance vector (DSP-Hygro). The resistant colonies were examined for the expression of PML after four hours of treatment in $ZnCl_2$ (150 μM) and subjected to a second cloning cycle by limited dilution. Inducible CHO clones were constructed in a similar manner. The monocytes were prepared by the method of Estaquier et al., 1997. The etoposide (used at 00 μM for 18 to 24 hours) was obtained from Biomol Research Laboratories, the zVAD (benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone, used at 250 μg/ml) was obtained from Bachem, and the rat IFNα was obtained from Access BioMedical. The human interferon α was provided by Schering-Plough. The anti-human PML protein antibodies are described in the article by Daniel et al., 1993. Western blotting experiments with endogenous rat PML protein were performed with the monoclonal antibody 5E10 which detects both rat PML and human PML.

The Bax protein was detected using a purified polyclonal rabbit serum directed against amino acids 80-98 (SC930 Santa Cruz). Another polyclonal rabbit antibody directed against another peptide (amino acids 11-30) (FC 793 Santa Cruz) and a Zymed monoclonal antibody gave similar results. p27 kip was detected by using a monoclonal antibody (Transduction Laboratories).

Evaluation of cell death

The cells were treated for 2 hours with 150 µM of $ZnCl_2$ (unless stated otherwise) in the presence or absence of foetal calf serum deactivated by heat, and the cells were then washed and incubated in a medium without $ZnCl_2$. The TUNEL test was performed according to the manufacturer's instructions (Boehringer Mannheim, *in situ* cell death detection kit), with the exception of the fixing stage (4% of formaldehyde in EBS phosphate buffer for 10 minutes). The content of cell DNA was evaluated by incubation for 10 minutes in 50 µg/ml of propidium iodide in the presence of 100 µg/ml Rnase A at 4°C. Analysis of the expression of phosphatidylserine on the external layer of cell membranes was performed using Annexiline-V-fluos labelling (Boehringer Mannheim) and loss of mitochondrial polarity was determined using rhodamine 123 (Molecular probes) according to the manufacturer's instructions. The samples were analysed on a FACScan analyser (Lysis II software; Becton Hickinson). For the cleavage of substrate by caspase, 5,106 cells were washed in PBS buffer for one hour at 4°C in 200 µl of lysis buffer (10 mM Hepes, pH 7.4, 2 mM EDTA, 2 mM DTT, 0.1 mM CHAPS). After centrifugation, 20 µl of supernatant and 180 µl of reaction buffer (100 mM Hepes, pH 7.4, 2% of glycerol, 5 mM DTT, 0.5 mM EDTA, 50 µm DEVD-pNA (Biomol Research Laboratories)) were mixed and the absorbance at 405 nm was measured after incubating for four hours at 37°C. The polyclonal antibody SA 252 is marketed by Biomol Research Laboratories.

EXAMPLE 1:

PML induces cell death independently of zVAD

The transfection of a PML (pSGS-PML) expression vector into different cell lines of fibroblasts strongly decreased the formation of sources. Since the PML protein was undetectable in the clones obtained from cells transfected by PML, the results imply that the PML exerts a major inhibiting effect either on the cell cycle or on the survival of the cells. To understand the mechanism based on this effect, a pool of embryonic rat fibroblasts (REFs) transformed by SV40T was transfected with a pKSmMT-PML plasmid, in which the expression of PML is under the control of a mouse metallothioneine promoter. Thereafter, three of the resulting REF(T)PML clones were studied, while three REFC(T) clones bearing the blank vector were tested as a control. The PML protein was detected by Western blotting two hours after exposure to $ZnCl_2$ (expression detectable from 50 M of $ZnCl_2$; exhibited a plateau at 150 μM of $ZnCl_2$) (Figure 1A). The expression of PML induced the synchronised cell death of the entire cell population, with kinetics varying from 46 hours at 50 μM of $ZnCl_2$ to 6 hours for 150 μM. In the three REF(T)PML clones and with 150 μM of $ZnCl_2$, morphological modifications were observed from three hours after induction. The cells were surrounded by a clear shrinkage of the cytoplasm (Figure 1B), became positive in a TUNEL test (Figure 1B), and were then progressively detached from the tray. They retained their capacity to exclude trypane blue, however. These modifications were associated with a modest content of subG1 DNA (Figure 1B), an externalisation of the membrane phosphatidylserine (Figure 1C) and a loss of the mitochondrial transmembrane potential (Figure 1C). Whilst similar modifications were observed in lapoptosis induced by the genotoxic agent etoposide, they were never found in REF(T) control cells treated with $ZnCl_2$ (Figures 1B and C). In contrast to the treatment by etoposide, the cell death induced by PML was not associated with nuclear morphological features typical of apoptosis such as the condensation of chromatine and nuclear fragmentation, even belatedly in the cell death process. Despite cleavage of the DNA (positive sub-GI (Figure 1B) and a loss of the viscosity of the DNA), cell death induced by PML was not associated with a graduation of the internucleosomal DNA, in accordance with the weak positive TUNEL signals (Figure lB).

Controls:

As REF(T) cells are cell lines transformed by SV40T, several experiments were performed in order to exclude any contribution by the "Big T" oncogene of the SV40 virus to the cell death induced by PML. Firstly, expression of the PML did not alter the expression or the location of SV40T in the REF(T)PML cells, nor did it degrade p53 or the liberation of p53 from the "Big T" oncogene of the SV40 virus. Secondly, in Hela or CHO cells which were transiently transfected with either a GFP-PML fusion protein or with GFP on its own, all the positive GFP-PML cells were progressively detached from the tray and died, in contrast to the positive GEP control cells. Thirdly, in CHO cells which were stably transfected with the plasmid pKSmMT-PML, induction by $ZnCl_2$ resulted, there also, in the death of clones expressing the PML protein. Finally, in REF cells expressing a thermosensitive SV40T mutant, degradation of the SV40T at 39.5°C did not affect the cell death triggered by the PML.

The induction of cell death can require a transcription *de novo* or can reflect the activation of pre-existing pathways. The REF(T)PML cells were firstly incubated with $ZnCl_2$ and with cycloheximide for two hours, thus permitting the synthesis of PML mRNA and not the translation thereof. The cells were subsequently washed and incubated with actinomycine D on its own, to permit the translation of PML mRNA but not mRNA neosynthesis. In this experiment, cell death was observed just the same as in the absence of inhibitor, showing that transcription *de novo* is not required. A death induced by PML does not necessitate and does not induce the transition to the S phase of the cell cycle. In fact, the PML always results in death in REF(T)PML cells that were blocked at the G1/S stage by treatment with aphidicoline. Moreover, exposure to BrdU at different times after induction by $ZnCl_2$ showed that replication of the DNA was not modified up to two hours, but was stopped after three hours, and that cell death was present in all phases of the cell cycle (Figure 1B).

EXAMPLE 2:

Arsenic promotes cell death triggered by PML

When REF(T)PML cells were treated with $ZnCl_2$ and 10^{-6} M As_2O_3, a rapid acceleration was observed in the morphological modifications associated with cell death. The cleavage of TEA as determined by TUNEL tests increased similarly (117% of positive cells for co-treatment with $ZnCl_2$ compared with 45% for $ZnCl_2$ only, whilst As_2O_3 on its own did not induce any increase in relation to the base level). The fact that arsenic increases the induction of cell death in parallel with the location of PML on nuclear bodies suggests that the location of PML near nuclear bodies is important for cell death.

EXAMPLE 3:

Death triggered by PML is not associated with caspase activation

It is known that the programmed occurrence of cell death involves the proteolytic activation of caspases, which induce phenotypical changes in apoptosis by the cleavage of nuclear and cytoplasmic proteins (Salvesen et al., 1997). The caspase inhibitor zVAD, which blocks apoptosis induced by etocoside, does not inhibit cell death induced by PML (Figure 10) and paradoxically even accelerates it (71% positive TUNEL signal with zVAD and $ZnCl_2$, compared with 45% with $ZnCl_2$ only). These observations imply that executants sensitive to zVAD are not required for cell death induced by PML. Moreover, CPF32 (caspase 3), which is the main caspase involved in apoptosis, appears not to be activated during cell death induced by PML, insofar as one of its substrates, PARP (poly(ADP-ribose)polymerase) remains uncleaved (Figure 2A). In contrast to etoposide, no significant cleavage of the colorimetric caspase substrates YVAD-pNa (class 1 caspase, Boeringer Mannheim) and DEVD-pNA (class 3 caspase, Boeringer Mannheim) could be detected after induction by PML (Figure 2B).

EXAMPLE 4:

Arsenic and zVAD maximise cell death induced by PML and interferons

Primary monocytes exposed to interferon α underwent a progressive cell death which resulted in the complete disappearance of the cell culture after seven days (Figures 3A and 3B). On the addition of zVAD with interferon α, death of the whole cell population was observed in 24 hours, in the absence of the nuclear fragmentation and chromatine condensation observed with interferon on its own (Figures 3A and 3B).

Little or no cell death cellular was observed with zVAD on its own for 20 days in most primary cultures (8/11) (Figures 3A and 3B). In three cultures out of eleven, zVAD on its own induced the death of part of the culture after seven days, these results probably reflecting the endogenous secretion of interferon. Similar results were obtained with other caspase inhibitors, such as DEVD.

The Table below illustrates cell death, as evaluated by the tunel test, of REF(T)P cells treated for two days with 1 000 U/ml of IFNα and 10^{-6} M As_2O_3 or zVAD.

	IFNα	
	-	+
Control	5 %	42 %
zVAD	5 %	60 %
As_2O_3	5.5%	63 %

In REF(T) cells, considerable synergy was found between either interferon α and zVAD, or interferon α and As_2O_3 (42% positive TUNEL signal for interferon α only, and 60% and 63% with zVAD and arsenic respectively).

zVAD increased the levels of expression of PML (Figure 4A), and arsenic increased its association with nuclear bodies although the total amount of PML had decreased. The similarity of the synergies of zVAD and of arsenic with the cell deaths triggered by PML and interferon suggests that PML is involved in cell death induced by interferon. Moreover,

interferon induces cell death with the same kinetics as 50 μM $ZnCl_2$, and they both induce similar quantities of PML protein (Figure 4B).

EXAMPLE 5:

The PML protein entrains the proteins Bax and p27 towards nuclear bodies

Bax and p27 are known as two products of proapoptotic genes. Double labelling with anti-PML and anti-Bax antibodies showed cytoplasmic labelling of the Bax protein and co-location of Bax proteins and endogenous nucleoplasmic PML on nuclear bodies, using three different antibodies directed against distinct portions of Bax. The superexpression of PML or PML/RARα in HeLa cells induced the recruitment of endogenous Bax on structures labelled with PML, strongly suggesting a contact between Bax and PML. Immuno-electronic microscopy demonstrated a preferential association of Bax on the periphery of nuclear bodies, as shown previously for PML. To ensure that the recruitment of Bax also occurs in cells which are not transfected, HeLa cells were treated with interferon and/or arsenic, which induce both the expression of PML and targeting towards nuclear bodies.

Interferon on its own recruited Bax and induced cell death. Arsenic promoted both the recruitment of Bax and cell death. As the total amount of Bax does not vary, these modifications result from the recruitment of Bax on cellular bodies.

Some practically similar observations were made with the inhibitor cdk p27/kip (cycline dependent kinase inhibitor), which is partly associated with nuclear bodies and is recruited by an IFN/arsenic treatment. The proteins Bax and p27 were thus identified as being new antigens bound to nuclear bodies involved in the induction of cell death.

CLAIMS

1. A use of at least one substance which promotes addressing of the PML protein to nuclear bodies and/or its stabilisation for the production of a drug intended to induce the death of undesirable cells, with the exception of leukaemia cells of acute promyelocytic leukaemia when said substance is a compound of arsenic.

2. A use of at least one substance selected from compounds of arsenic, compounds having the same biological properties as arsenic, and caspase inhibitors and/or substrates for the production of a drug intended to induce the death of undesirable cells, with the exception of leukaemia cells of acute promyelocytic leukaemia when said substance is a compound of arsenic.

3. A use according to either one of claims or 2, wherein said substance is associated with the PML protein and/or with an agent which induces superexpression of the PML protein,
the administration of said substance and the administration of the PML protein and/or of said agent which induces superexpression of the PML protein being simultaneous or sequential.

4. A use according to claim 3 in which said agent which induces superexpression of the PML protein is an interferon such as interferon α or β.

5. A use according to any one of claims 1 to 4, wherein said substance is arsenic trioxide.

6. A use according to any one of claims 1 to 4, wherein said substance is zVAD.

7. An *in vitro* method of inducing the death of undesirable cells, comprising placing the undesirable cells in contact with a substance selected from compounds of arsenic, compounds having the same biological properties as arsenic, and caspase inhibitors and/or substrates.

8. A method according to claim 7, wherein said substance is associated with the PML protein and/or with an agent which induces superexpression of the PML protein, preferably an interferon.

9. A pharmaceutical composition containing

1) either at least one inhibitor and/or a caspase substrate, associated with:

- at least one compound of arsenic or a compound having the same biological properties as arsenic,
- and/or the PML protein
- and/or at least one agent which induces superexpression of the PML protein, such as an interferon,

in the presence of a pharmaceutically acceptable vehicle;

2) or at least one compound of arsenic or a compound having the same biological properties as arsenic associated with the PML protein and/or with at least one agent which induces superexpression of the PML protein, such as an interferon, in the presence of a pharmaceutically acceptable vehicle.

10. A kit comprising

a) - a pharmaceutical composition (1) containing at least one inhibitor and/or caspase substrate, in association with a pharmaceutically acceptable vehicle;
and/or a pharmaceutical composition (2) containing at least one compound of arsenic or a compound having the same properties as arsenic, in association with a pharmaceutically acceptable vehicle; and

b) - a pharmaceutical composition (3) containing the PML protein in association with a pharmaceutically acceptable vehicle;

- and/or a pharmaceutical composition (4) containing at least one agent which induces superexpression of the PML protein, such as an interferon, in association with a pharmaceutically acceptable vehicle;

said pharmaceutical compositions being intended for simultaneous or sequential administration.